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Journal of Hepatology 49 (2008) 145–148

Journal of Hepatology

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Letters to the Editor

Is bacterial DNA a surrogate marker of bacterial translocation in cirrhosis?

To the Editor:

I read with interest the excellent clinical study by Gonzalez-Navajas and colleagues [1]. Following their previous study showing that about 40% of patients with advanced cirrhosis had bacterial DNA in blood and ascites and indicating that repeated episodes of bacterial translocation (BT) evidenced by the detection of same bacterial DNA in consecutive blood samples are highly suspected in these patients [2]. In this study the authors investigated whether the presence of bacterial DNA is associated with pro-inflammatory cytokine and nitric oxide responses independently of the endotoxin pathway. Forty-seven (33%) of 141 patients with cirrhosis and non-infected ascites showed the simultaneous presence of bacterial DNA in blood and ascites. Among these 47 patients, the first eight with bacterial DNA from Gram-positive cocci, the first eight with DNA from Gram-negative bacteria, and the first 16 patients without bacterial DNA were included in the study. Serum endotoxin and lipopolysaccharide-binding protein levels (LBP) in patients with Gram-positive bacterial DNA were similar to those in patients without bacterial DNA. Regardless of lower LBP and endotoxin levels in patients with Gram-positive bacterial DNA compared to those in patients with Gram-negative bacterial DNA, serum levels of tumour necrosis factor (TNF)-α and interleukin-6 (IL-6) in patients with Gram-positive bacterial DNA were significantly higher than those in patients without bacterial DNA and equivalent to patients with Gram-negative bacterial DNA. The authors concluded that the detection of bacterial DNA allows the identification of BT in patients with advanced cirrhosis, which is associated with increased inflammatory responses independently of endotoxin signaling.

The results of this study are consistent with the suggestion by a recent review [3] that Gram-positive bacteria or their components play an important role in the inflammatory response in patients with cirrhosis because the expression of Toll-like receptor (TLR)-2, which responds predominantly to cell wall components of Gram-positive bacteria, has repeatedly been shown to be upregulated, whereas the expression of TLR-4, a specific transducing receptor for endotoxin, is unaltered or downregulated in cirrhosis. I would like to address three questions. First, I would like to ask the authors whether the relationship between the detection of bacteria by blood culture and the identification of bacterial DNA by polymerase chain reaction has previously been examined in cirrhotic or other patients. It has been reported that there were 16.6% false-positive results when DNA was isolated from healthy volunteers with a commercially available kit [4]. Contamination can originate from mastermix reagents and the DNA extraction procedure. Second, the values of TNF-α and IL-6 reported in this study (mean TNF-α: 324.93 μg/mL, mean IL-6: 294.96 μg/mL in patients with bacterial DNA) are more than 1,000,000-fold higher than those of our data and the results of a recent study (mean TNF-α: 90.9 pg/mL, mean IL-6: 84.8 pg/mL in patients with decompensated cirrhosis) [5]. I think there is an error in the conversion of the units. Third, I would like to ask the authors whether the difference (<0.3 EU/mL) in endotoxin concentrations between the patients with Gram-negative bacterial DNA and those with Gram-positive bacterial DNA is clinically relevant. The biological activity of endotoxin is not only dependent on endotoxin and LBP concentrations but also influenced by soluble CD14 levels, lipoprotein concentrations, the expression of membrane CD14, TLR-4, MD-2, and the activity of transcription factors.

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doi:10.1016/j.jhep.2008.04.001

Bacterial DNA translocation in patients with cirrhosis: Reply

To the Editor:

We thank Fujita and colleagues for their comments and this opportunity to discuss the questions raised regarding our recent investigation on bacterial DNA translocation in patients with cirrhosis.

Our main line of research is focused on the study of bacterial translocation (BT) in patients with decompensated cirrhosis. Approximately, 30% of these patients show the simultaneous presence of bacterial DNA (bactDNA) in blood and ascitic fluid (AF) [1,2] and a recent study in an experimental model of CCl_4-induced cirrhosis with ascites positively correlated this finding with the identification of bactDNA in mesenteric lymph nodes [3]. However, and related to the first question, we recently conducted a study in which bactDNA detection and the associated inflammatory response was evaluated in patients with spontaneous bacterial peritonitis (SBP). In this study, a total series of 22 patients with SBP was analyzed. All 22 cases showed the presence of bacterial genomic fragments as revealed by PCR of the 16SrRNA gene. Identifications by DNA sequencing analysis in patients with culture-positive SBP corresponded to those isolated by microbiological culture in all cases, except for one patient that showed a *Staphylococcus aureus* by sequencing analysis and a *Streptococcus pneumoniae* by microbiological culture [4].

Regarding experimental procedures, control experiments are normally run over all DNA extraction Kit solutions as well as PCR mastermix reagents. Specificity experiments are always completed with a 10 pg-spike of *Escherichia coli* DNA to determine the presence of inhibitors in the media. In addition, all solutions and reagents are handled in sterile air-flow chambers previously irradiated with UV light, and sterile plastic material and filter tips are used all along the technique of DNA isolation and amplification. Finally, in all PCR reactions, two negative controls are always run: one with sterile water instead of sample templates and one with PCR mastermix alone. We are aware of the study by Heininger A et al. in which, as pointed out by Fujita T, 16.6% false-positive results are present when DNA is isolated from healthy volunteers with a commercially available kit [5]. DNA isolation kit used in that study (Puregene Whole Blood Kit, Biozym Diagnostics) is not the same as the one we usually handle (QiAmp Blood Minikit, QIAgen). Also, the set of primers used to perform PCR reactions are also different in that study. Therefore, it is difficult for us to compare or discuss on someone else's reported contamination rates.

A significantly increased cytokine response is present in patients with presence of bactDNA [6,7]. Despite this increment, values have never reached μg/ml levels. This is, as suggested, a typing mistake. All cytokine determinations were measured in pg/mL. We apologise for this confusion and thank Dr. Fujita and colleagues for their appreciation. Indeed, in the previously mentioned study on the inflammatory response in patients with advanced cirrhosis and ascites, extended results on several cytokine levels can be followed in different clinical settings.

Endotoxin is a difficult to measure parameter. LPS traces can be detected in serum from patients with presence of Gram-positive microorganisms. We consider that reported differences in endotoxin levels simply reflect intrinsic characteristics of both subgroups of bacterial species and do not allow discussion about its biological activity. For this purpose lipopolysaccharide binding protein (LBP) measurement has been proposed instead [8]. However, endotoxin, DNA or peptidoglycan, among others bacterial products, stimulate the innate immune system and induce the liberation of proinflammatory cytokines and nitric oxide, and these factors indeed have clinical consequences. The detection of high levels of endotoxin or the above-mentioned bacterial products identify a subgroup of patients with evidences of bacterial translocation, and this may affect prognosis [9].